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Sol Gel Assisted Microwave Synthesised Hydroxyapatite Nanostructure using Biowaste Musselshell as A Calcium Source

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Abstract

The common waste material from fish industry is Mussel shells which is normally disposed in land areas. The main composition of mussel shells is Mussels and aragonite. Hence it is considered as a by product for producing nanomaterials. Nano Hydroxyapatite [n-HAp, $Ca_{10}(PO_4)_6(OH)_2$] has been prepared by microwave irradiation method using Mussel shell and Orthophosphoric acid as precursors at normal room temperature. Powder XRD results suggest that HAp are found to be hexagonal, P63/m symmetry, well matched with JCPDS card no: 09-0432. Restriction of particle growth was due to the substitution of capping agent EDTA while precipitation. FTIR predicts the presence of C-O and P-O stretching. The characterized sample is uniformly distributed, crystalline and elongated in nature. The morphology was identified to be spherical shaped by SEM micrographs with diameter of around 40 – 32 nm. EDAX reveals that it has standard Ca/P ratio 1.6. TEM reveals rod shaped Morphology.

Keywords: *Biomedical applications; Biomaterials; Hydroxyapatite; Mussel shell; Precipitation.*

1. INTRODUCTION

The natural and synthetic materials which are directly applied in the bio environment is Biomaterials. The biomaterial fabrication involves restoration of body tissue functioning, mechanical properties, design and its biocompatibility (Haresh.M.Pandya, 2012). The biomaterial should possess the following mechanical properties namely elasticity, wear resistance, yield stress, toughness and ductility etc,. It should be formed in many shapes, low cost and easy availability. They are applied in fabrication of implant devices such as hip joint prosthesis, joint prosthesis, dental implants, etc (Amin shavandi *et al.* 2015). Biomaterials are non-toxic, biocompatible, bioactive, integrate into living tissue. Hydroxyapatite, a calcium phosphate material in crystalline form $Ca_{10}(PO_4)_6(OH)_2$. bone is a fiber-reinforced composite, teeth is calcium orthophosphate-based calcified phase. Bone consist of approximately 8wt% water, 22wt% protein and 70wt% minerals. The mineral component of the bone is a form of calcium phosphate which present the main mineral reservoir for the body. Among all biomaterials calcium phosphate are attractive biomaterials owing to its excellent biocompatibility and its non- toxicity. It is a very good drug delivery carrier and possesses Favorable biodegradability and biocompatibility properties. Soluble and less toxic than silica, Quantum dots, carbon nanotubes and magnetic particles. Calcium phosphate based system and particularly those with Ca/P molar ratio close to the one of Hydroxyapatite are negligibly soluble in blood which is by itself supersaturated with respect to Hydroxyapatite. Teeth has two bio minerals namely enamel and dentin. (Among tathe *et al.* 2010, Sahil Jalota *et al.* 2004]. The crystal known forms are HAp is monoclinic(P21/b) and hexagonal (P63/m) with $Ca_{10}(PO_4)_6(OH)_2$.Hydroxyapatite shows excellent antibacterial properties when incorporate with silver ions due to interaction of silver ions with thiol groups. (Haded Alobeedallah *et al.* 2011). Sol–gel offers advantage of molecular-level mixing of reactants, improving the chemical homogeneity of the resulting powder (Kapoorseema *et al.* 2011, Sanosh et at. 2009). Low- temperature formation and fusion of the prepared crystals are other notable advantages of the sol–gel process. Microwave synthesis of nano phase ceramic materials is relatively a new class of method, which has recently gained interest in the nanomaterial synthesis. The introduction of organic modifier like CTAB, citric acid, EDTA controls the shape and size of HAp nanoprticles by the microwave irradiation method (Sopyan *et al.* 2011). EDTA is the efficient capping

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agent during the synthesis of Hydroxyapatite nanoparticles (Mark I Jones *et al.* 2011).

2. SYNTHESIS OF HA USING BIOGENIC SOURCES

The biogenic sources based HAp is well accepted by human body due to its similar physicochemical property. Extraction of bio minerals from biowastes like bovine bones, fish-scales, and fish bones is mostly preferred for preparing HAp. This is a waste from worth method.

Eggshells are composed mainly of calcium carbonate(~95-97%) , a calcium precursor in the synthesis of HA .This can be achieved in many ways. First The eggshells are heated in a furnace to remove organic matter and converts $CaCO_3$ to CaO. CaO is exposed to atmosphere to get $Ca(OH)_2$. (Haresh M. Pandya *et al.* 2013). This calcium hydroxide when treated with phosphorus precursor it produces Hydroxyapatite HA . Similarly eggshells can be treated by nitric acid or hydrochloric acid to get calcium nitrate or calcium chloride. Orthophosphoric acid and calcium phosphate act as best phosphate precursors to produce monophase HAp with plate like structure (Gobi *et al.* 2013).

Mussel shells are the main waste material of fish industry. Disposing of mussel shell causes environmental problems like odours while treatment and transportation is a risk factor. Mussel shells has about 55% of its total weight as 95%-99% aragonite .Hence applied in the synthesis of HAp. Limited research has been done in this area, to produce HAp from mussel shells. The nanocrystalline HA produced from waste mussel shells using a rapid microwave irradiation method (G.C.Koumoulidis *et al.* 2003) where mussel shells were converted into rod like nanocrystalline HA particles of 30-70 nm long using 0.1 M EDTAas a chelating agent (Kanchana *et al.* 2014) for 30 min after an appropriate pre treatment and an irradiation step in a microwave with a power of 1.1 kW .The preparation of hydroxyapatite using mussel shell, where raw shells were first calcined to produce lime (CaO) and then it was reacted with phosphate by simple Sol gel process at a low temperature (Kalaiselvi *et al.* 2015).

An attempt has been made to synthesize pure and biocompatible HAp powder by using Mussel as the calcium source. Mussel shell is composed of calcium carbonate (94%), calcium phosphate (1%), organic matter (4%) and magnesium carbonate (1%). At the temperature 900°C, the shells transformed into calcium oxide by releasing carbon dioxide (CO_2) according to the following equation:

$$CaCO_3 \rightarrow CaO + CO_2\uparrow$$

The CaO obtained from the mussel shells was then converted into HAp in a phosphate solution. Due to rich calcium content in Mussel shell the present work is carried out. (Govindan Suresh Kumar *et al.* 2017). The scope of the present work is to adopt a simple and rapid sol gel assisted microwave irradiation method for recycling the mussel shell biowaste.

2.1. Chemicals

The chemicals used were Ortho phosphoric acid (H_3PO_4, 99%), ethylene diamminetetraacetic acid disodium salt dihydrate ($C_{10}H_{14}N_2Na_2O_8.2H_2O$, 99%) sodium hypochlorite (NaOCl, 97%)and disodium hydroxide pellet (NaOH, ≥ 97 %) obtained from Merck. All reagents were used without further purification. Distilled water was employed as the solvent.

2.2 Synthesis

The Mussel shell was collected from nearby water areas. The surface impurities were removed by washing the shells with distilled water. The washed shells were grinded into fine powder using pestle and mortar. The organic contaminants are removed by dispersing the powder in Sodium hypochlorite solution.. Then wash using distilled water and dry in hot air oven for 5h at 110°C. Take one gram of Mussel shell powder and dissolve in 0.1M EDTA to get Ca-EDTA complex. To the above solution add 0.6M of H_3PO_4 solution slowly while stirring for 30 min. Sodium hydroxide was used to adjust the pH 13. The mixture was allowed to age for 24hrs.The aged solution was washed with distilled water to get a white precipitate. Then the obtained precipitate was placed in domestic microwave oven (2.45 GHz, 700W) and irradiated with microwave for 15 min to get a final mixture. Further it is grinded to get a HAp nanopowder.

2.3 Characterization techniques

2.3.1 X-Ray diffraction analysis

X-ray diffraction is the advanced analytical technique used for identifying the crystal size, unit cell dimensions, phase identification and atomic arrangement. XRD is based on Bragg's law and related to constructive interference of monochromatic X-ray and a crystalline sample. The lattice parameters (*a* and *c*) of the sample were calculated from the equation for hexagonal system using the method of least squares

$$\frac{1}{d^2} = \frac{4(h^2 + hk + k^2)}{3a^2} + \frac{l^2}{c^2}$$

Where *d* is the spacing between the planes in the atomic lattice which was calculated according to Bragg's equation

$$2d \sin\theta = n\lambda$$

The volume *V* of the hexagonal unit cell was determined from the following relation.

$$V = \frac{\sqrt{3}}{2} \times a^2 \times c$$

2.3.2 Fourier Transformation Infra red spectroscopy (FT- IR)

Fourier Transform Infrared method is useful technique for materials analysis. The prism is used to separate visible light into different colours emitted from Infra red source. The detectors are used to detect the frequency passed through the sample in the region 4000-400 cm^{-1}. The graph is drawn for wave number and Transmittance.

2.3.3 Scanning Electron Microscopy(SEM) with EDAX Analysis

A scanning electron microscope (SEM) helps to predict the composition, surface topography, crystalline structure, orientation of the sample. It can also perform the selected point locations which help in determining the chemical composition of the sample. SEM and EDAX are performed using JEOL make JSM6390 microscope. High- resolution Cathode Ray Tube is also used in photography.

2.3.4 Transmission Electron Microscopy Analysis

TEM can reveal the morphology, particle size, electronic structure and crystal orientation of the sample. The sample undergoes TEM using JEOL JEM - 2100 transmission electron microscope under different magnification.

3. RESULTS AND DISCUSSION

3.1 X-Ray diffraction Analysis

Characterization of HA synthesized using mussel shell as a calcium source

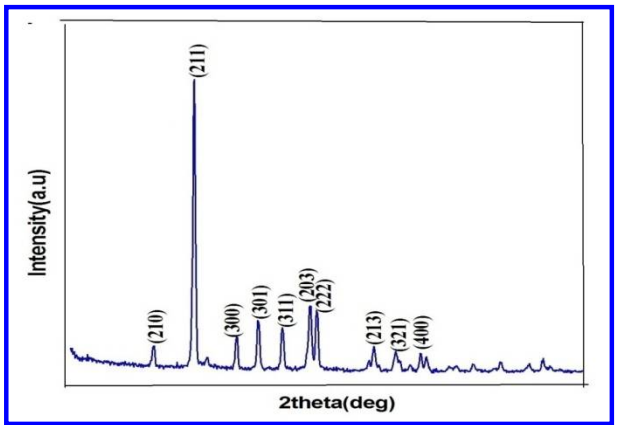

Fig. 1: XRD pattern of HA obtained using mussel shell as a calcium source.

Fig. 1 shows the XRD pattern of synthesized HAP powder. The X-ray difraction patterns obtained matches well with standard data of HAP (JCPDS File No: 09-0432) which indicates the presence of pure HAP. The lattice constants was calculated as

$a = b = 9.9110$ Å,
$c = 6.1051$ Å and
c/a= 0.6159

The Unit cell volume were calculated as $V = 531.81$ Å ^{3}. The Crystallite size is 28 nm. This restriction occurs due to the presence of capping agent EDTA.The high intense peaks indicate the crystalline nature of Hydroxyapatite.

3.2 Fourier Transformation Infra red spectroscopy

FT-IR also used is to examine the positions of carbonate in HA structure.

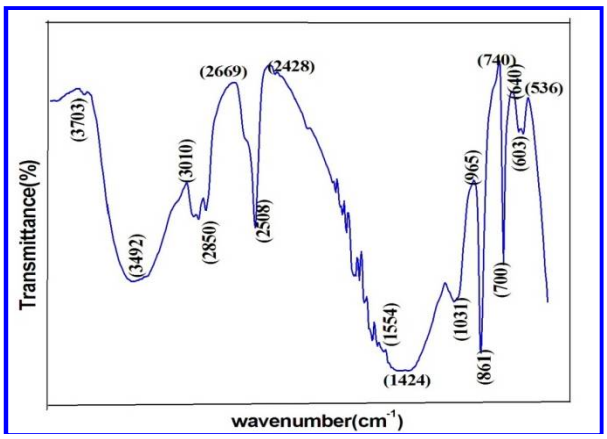

Fig. 2: FT-IR spectrum of HA obtained using mussel shell as a calcium source

Fig. 2 reveals the FTIR image of the synthesised HAp.The presence of functional groups are analysed by FTIR spectra. The FTIR image reveals the characteristic frequencies of . P-O bending vibration at 536cm^{-1}. P-O stretching vibration was found in 861 cm^{-1} . The wave number 1424 cm^{-1} indicates C-O stretching vibration. Stretching peak value of 3703 cm^{-1} is assigned to hydroxyl O-H functional groups. The adsorbed H_2O in the sample is observed at 1554, 1666 cm^{-1} and 3492 cm^{-1}.

The stretching and Bending vibration shows the chemical bonds present in the Hydroxyapatite. The characteristic $PO43^{-}$ (ν_4) vibrations of HA is present at 603 cm^{-1} . The broad band expanding from 1605 to 3397 cm^{-1} is appear to the ν_3 and ν_1 stretching modes of the H_2O molecules. The additional peaks at 861 cm^{-1} (ν_2), 1424 cm^{-1} (ν_3) and 1461 cm^{-1} (ν_3) reveals the presence of carbonated HAp type B.

3.3 Scanning Electron Microscopy

The pictorial representation of SEM is depicted in the fig. The size and shape of the HAp was

visualized by SEM. The Pure Hydroxyapatite exhibits conglomerated structure ranges between 71 to 131 nm. The figures are represented in different magnification. The conglomerated images reveals the presence of Hydroxyapatite.

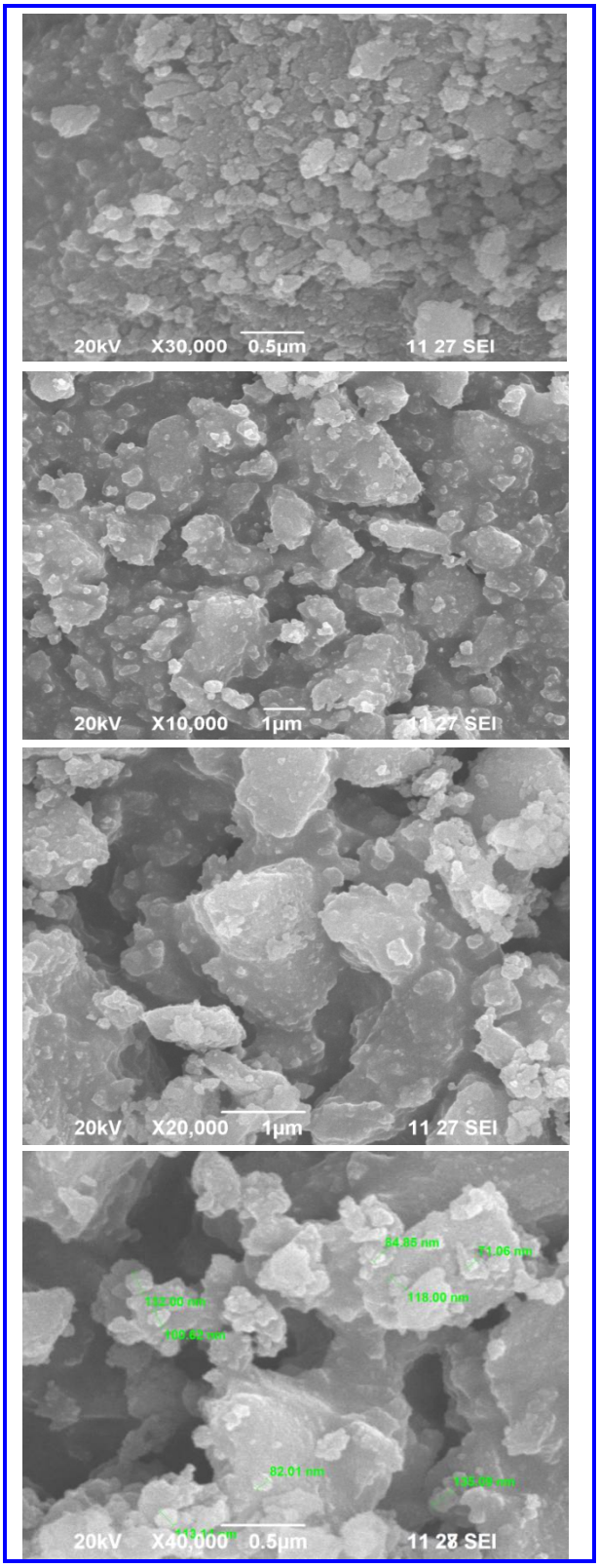

Fig. 3: SEM image of Hydroxyapatite synthesised from Mussel shells.

3.4 EDAX (Energy Dispersive X-Ray Spectroscopy)

EDAX analysis reveals the elemental composition and purity of the synthesised sample**.** The atomic weight percentage of the elements are Ca-17.39, P-10.51, O-72.10.The Ca/P ratio of pure Hydroxyapatite synthesised from Mussel shells is 1.69 which is close to the expected value (1.67). The small difference in the value can be attributed to impurities in the mussel shells.

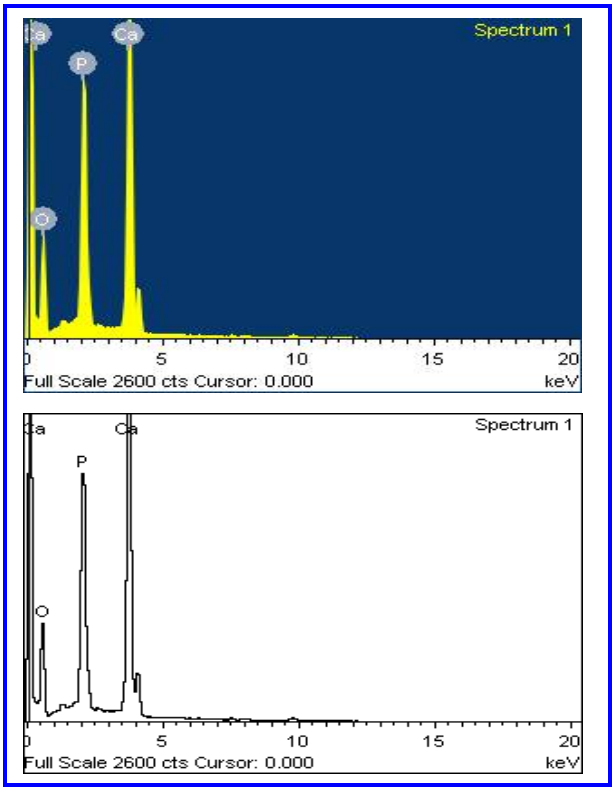

Fig. 4: Energy Dispersive X-Ray Spectroscopy of Hydroxyapatite synthesised from Mussel shells

3.4 Transmission Electron Spectroscopy analysis

The Transmission electron spectroscopic analysis of Hydroxyapatite. from Mussel shells with the magnification of 500nm and 200nm are displayed in the figure.

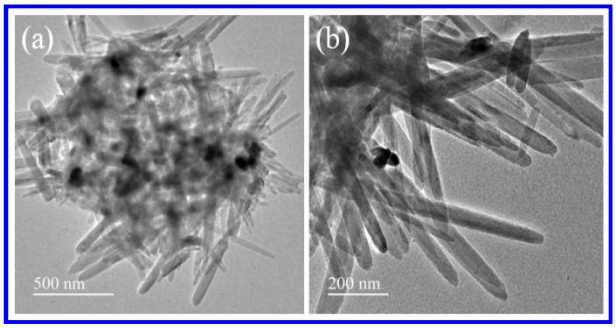

Fig. 5: TEM images of Hydroxyapatite obtained using mussel shell as a calcium source (a) lower magnification and (b) Higher magnification.

The image shows needle shape in lower magnification and rod shape in higher magnification**.** Thus the rod shaped morphology of the sample clearly proves that the sample can be applied in the field of Orthopedics and Dentistry.

4. CONCLUSION

Solgel assisted microwave irradiated Hydroxyapatite nanorods have been synthesized successfully by using mussel shell as a calcium source involving EDTA as a capping agent. X-ray diffraction analysis confirmed the crystal size, Lattice parameters, and unit cell volume. The final product was identified to be pure Hydroxyapatite with hexagonal structure having $a = b = 9.9110$ Å , $c = 6.1051$ Å and unit cell volume as $V = 531.81$ Å ^{3}. FTIR showed the presence of Phosphate and Hydroxyl groups in the sample. The SEM results showed that the formation of conglomerated structure in the sample.EDAX reveals the composition of the elements. Thus by recycling and waste management method mussel shells can be utilized as source for HAP synthesis. Mussel shell based HAP is an inexpensive ceramic biomaterial can be produced in masses.

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